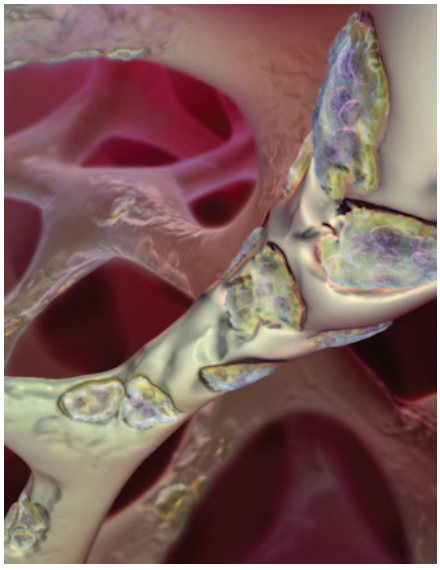
be needed to show whether this new BAT regulating mechanism is also active in humans and whether it could be explored as a relevant therapeutic target for metabolic and obesity disorders. —*VA*

■ BONE

Autoantibodies target bone

Autoantibodies against proteins marked with a type of post-translational modification called citrullination are found in people with rheumatoid arthritis and are one of the strongest risk factors for bone destruction in this disease. A recent study now directly links the formation of these antibodies to bone loss in rheumatoid arthritis, indicating that the autoantibodies act on osteoclasts, the bone cells responsible for bone resorption (*J. Clin. Invest.* **122**, 1791–1802).



Gary Carlson / Photo Researchers, Inc.

Ulrike Harre *et al.* found that the presence of anti–citrullinated protein antibodies (ACPAs) is associated with increased amounts of serum markers of bone resorption in people with rheumatoid arthritis. They affinity-purified autoantibodies against mutated citrullinated vimentin from patients; the production of these antibodies is highly specific to people with rheumatoid arthritis. *In vitro*, these purified ACPAs bound to the surface of osteoclast progenitors and induced their differentiation into mature osteoclasts and also increased osteoclast-mediated bone resorption.

The authors then injected the purified ACPAs into immunodeficient *Rag1*$^{-/-}$ mice, leading to systemic bone loss. This bone loss seemed to be mediated through an increase in osteoclast precursor numbers and increased osteoclast differentiation in response to ACPAs. These effects may depend on tumor necrosis factor-α, as the authors observed increased amounts of this inflammatory cytokine, which is known to induce osteoclastogenesis, in the mice treated with ACPAs.

This work thus provides new insights into the interactions between the immune system, inflammation and bone in rheumatoid arthritis. —*MS*

■ GENETICS

Somatic mutations in brain

A new study highlights an intriguing role for somatic mutations restricted to the brain in a developmental brain disorder, hemimegalencephaly (HMG) (*Neuron* **74**, 41–48).

HMG is an epileptic brain disorder characterized by the enlargement and malformation of one hemisphere of the brain. Given this regional selectivity of the deformation, it has been suggested that HMG may be caused by a somatic mutation limited to the brain, and Annapurna Poduri *et al.* now provide evidence to support this hypothesis.

The authors analyzed resected brain tissue samples from eight individuals with HMG and found that two of the samples showed trisomy of chromosome 1q, but, in one of the individuals, the blood sample did not show this genetic aberration. Of the genes on chromosome 1q, Poduri *et al.* suggest *AKT3* as a strong candidate gene for HMG, because *AKT3* deletions have been previously associated with microcephaly in humans and mice. Moreover, somatic activating mutations in the related genes *AKT1* and *AKT2* have been associated with human overgrowth syndromes. The authors also found a somatic activating mutation of AKT3 (E17K) in the brain of one individual with HMG.

These results suggest that somatic mutations in the brain could have an important role in neurogenetic disease, although the mechanisms by which they occur remain to be determined. They also bring up an interesting parallel between somatic mutations in cancer and in brain disease: somatic *AKT3* mutations have also been identified in cancer, but none of the individuals studied by Poduri *et al.* had any form of cancer, suggesting that *AKT3* mutations may result in different outcomes in different cellular contexts. —*MS*

Written by Victoria Aranda, Eva Chmielnicki, Alison Farrell, Carolina Pola and Meera Swami

New from NPG

Apolipoprotein E controls cerebrovascular integrity via cyclophilin A
Bell, R.D. *et al. Nature* doi:10.1038/nature11087 (16 May).
The authors provide a mechanistic link between the *APOE4* gene and blood-brain–barrier defects by showing that *APOE4* expression in mice activates a pathway in pericytes involving cyclophilin A (CypA) activation. This leads to neuronal uptake of blood-derived neurotoxic proteins. Thus, CypA may be a potential target for treating APOE4-mediated neurovascular injury, such as in Alzheimer's disease.

PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue T_{reg} cells
Cipolletta, D. *et al. Nature* doi:10.1038/nature11132 (16 May).
This study uncovers a new aspect to the action of the thiazolidinedione drug pioglitazone, used in the treatment of type 2 diabetes. The authors show that a population of regulatory T cells in the visceral fat are controlled by peroxisome proliferator–activated receptor γ (PPAR-γ) and that the expression of PPAR-γ in these cells is important for the insulin-sensitizing activity of pioglitazone.

Detectable clonal mosaicism and its relationship to aging and cancer
Jacobs, K.B. *et al. Nat. Genet.* doi:10.1038/ng.2270 (6 May).

Detectable clonal mosaicism from birth to old age and its relationship to cancer
Laurie, C.C. *et al. Nat. Genet.* doi:10.1038/ng.2271 (6 May).
Two new studies find mosaicism for large chromosomal abnormalities in peripheral blood samples from individuals in the general population. The frequency of these genetic changes increases with age and is associated with an increased risk of subsequently developing a hematological cancer.

Adolescent impulsivity phenotypes characterized by distinct brain networks
Whelan, R. *et al. Nat. Neurosci.* doi:10.1038/nn.3092 (29 April).
The authors examine the activation of brain networks in adolescents with attention-deficit hyperactivity disorder (ADHD) and those who had used drugs or alcohol using functional magnetic resonance imaging. They find that although both ADHD and substance abuse are associated with increased impulsiveness, different brain networks were activated in response to a task testing impulsiveness in these two contexts.

J Rheumatol. 2001 Jul;28(7):1492-5.

Autoantibodies to osteopontin in patients with osteoarthritis and rheumatoid arthritis.

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Abstract

OBJECTIVE: Osteopontin (OPN), eted mainly from chondrocytes, is suggested to be involved in the ossification and remodeling of bone and also in regulation of cytokine profiles. We investigated whether patients with osteoarthritis (OA) and rheumatoid arthritis (RA) display autoimmunity against OPN.

METHODS: Recombinant human OPN (rhOPN) was prepared as a fusion protein with beta-galactosidase using E. coli. Serum samples from patients with OA or RA and from age matched healthy donors were tested for autoantibodies to rhOPN using ELISA and Western blotting. Reactivity of the same samples to purified native human OPN (nhOPN) was investigated by ELISA separately, to evaluate conformational epitopes.

RESULTS: By ELISA, autoantibodies to rhOPN were found in one (0.95%) of 105 patients with OA and 2 (2.3%) of 88 patients with RA. These autoantibodies to rhOPN were confirmed by Western blotting. In contrast, 11 (9.5%) of 105 OA serum and 13 (15%) of 88 RA serum samples reacted to nhOPN. The anti-OPN positive RA patients showed high serum levels of rheumatoid factor and C-reactive protein and accelerated erythrocyte sedimentation rate compared to the anti-OPN negative group, although the differences did not achieve statistical significance.

CONCLUSION: Our data showed that OPN is one of the autoantigens in OA and RA. Preferential recognition of nhOPN to rhOPN indicates that major epitope(s) of OPN would be conformational. Clinically, existence of the anti-OPN antibodies may be linked to disease severity in RA.

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HIGH TITERS OF ANTI-BONE AUTOANTIBODY ARE ASSOCIATED WITH OSTEOPROSIS OF PATIENTS WITH CELIAC DISEASE.

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BACKGROUND/AIM The pathophysiological mechanism(s) inducing osteopenia and osteoporosis in celiac disease patients are still unknown. We recently detected that sera of untreated patients present an IgA type autoantibody (anti-bone) against autoantigen(s) located in the pericellular matrix of fetal rat bones. Our aim was to establish the relation of the anti-bone antibody with the presence of bone impairment of patients. METHODS: We evaluated serum samples from 18 patients with celiac disease (16 female; median age: 38 years; range: 24-65 yr) with different degree of bone mineral density (BMD) at diagnosis. While 7 patients had normal BMD (> -1 z-score), 4 presented osteopenia (1 to 2 z-score) and 7 had osteoporosis (< -2 z-score). Patients with osteoporosis were reevaluated after treatment (median time: 13 mo.). Anti-bone autoantibody detection was performed using indirect immunofluorescence on 5μm thin cryostat sections of fetal (20 day pregnancy) rat tibia. Serum samples were tested at increased dilutions from 1:5 until a completely negative immunofluorescence could be demonstrated. RESULTS: At diagnosis, patients with osteoporosis evidenced significantly greater titers of anti-bone antibody (median 1:160; range 1: to 1:1280) than patients with BMD grater than 2 z-score ($p<0.02$). Patients with normal BMD were negative for anti-bone antibody (n=2) or presented very low titers (3 were +ve at 1:5 and 1 was +ve at 1:40). All patients with osteopenia had +ve titers (3 were +ve at 1:5 and 1 at 1:40). Patients with osteoporosis had significantly higher titers (2 at 1:5; 4 at 1:160 and 1 at 1:1280) compared with other groups. Patients with osteoporosis presented a significant reduction of titers after treatment ($p<0.05$). A non-significant correlation was observed between BMD and serum titers of anti-bone antibody (r: 0.298). CONCLUSIONS: We show evidence that most patients with celiac disease have a circulating autoantibody against intercellular structures of fetal rat bones. Higher titers of this anti-bone autoantibody correlates significantly with the most sever bone impairment and reduced significantly by treatment. We suggest that anti-bone autoantibody could be involved in the pathogenesis of bone disease associated with celiac disease. Further studies are necessary to identify and characterize antigen(s) eliciting anti-bone autoantibody.

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EXPRESSION OF RECEPTORS FOR VASOAVTIVE INTESTINAL PEPTIDE, SECRETIN AND SOMATOSTATIN IN COLORECTAL CANCER.

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Expression of receptors for vasoavtive intestinal peptide, secretin and somatostatin in colorectal cancer *C Tang[1], I Biemond[2], HW Verspaget[2], CBHW Lamers[2]* Dept of Gastroenterology, The First Hospital, Chongqing University of Medical Sciences, Chongqing, P R China[1]. Dept of Gastroenteology- Hepatology, Leiden University Medical Center, Leiden, The Netherlands[2]. Increasing interest in the application of gut peptide analogues or antagonists in the diagnosis and treatment of colorectal cancer necessitates a better insight and characterization of receptors for gut peptide in the development and metastasis of colorectal cancer. This study visualized and characterized the receptors for vasoactive intestinal peptide (VIP), secretin and somatostatin (SST) in the development and metastasis of colorectal cancer with storage phosphor autoradiography. Receptors for these three peptides were demonstrated in tumor-free colon and colon tumors. A decrease in affinity of VIP receptors was shown in the sequence from tumor-free colon (Kd = 0.93 nmol),colonic adenoma (Kd = 1.98 nmol), carcinoma (Kd = 2.06 nmol)to liver metastases (Kd = 3.30 nmol). An up-regulation of receptors for SST or secretin was found in colonic liver metastases. The binding of ^{125}I-SST could be inhibited by octreotide in tumor-free colon but not in colonic tumors.It indicated that tumor-free colon expressed SST receptor (SSTR)subgroup containing SSTR-2, SSTR-3 or SSTR-5 but colon tumors expressed SSTR-1 or SSTR-4. An up-regulation of SSTR-1 or SSTR-4 in colonic liver metastases may present a growth advantage in colorectal cancer. In conclusion, co-expression, although reverse in characteristics, of receptors for VIP and secretin was observed in the development and metastasis of colonic carcinoma. The difference observed in the SST receptor subgroup in colon tumors compared to tumor-free colon as well as the up-regulation of SST receptors in colonic liver metastases may have clinical implications.

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THE EFFECTS OF RABEPRAZOLE ON ECL CELLS AND PARIETAL CELLS IN RAT -COMPARISON WITH OMEPRAZOLE-.

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Rabeprazole (RPZ) is more potent in inhibiting the H^+-K^+-ATPase (HKA) and *in vivo* gastric secretion in dogs than omeprazole (OPZ) and lansoprazole. But this compound is dissociated from the acid pump by the reaction of glutathione, generating faster recovery from the initial acid inhibition. This study was carried out to investigate the effects of RPZ on ECL cells and parietal cells and compare them with those of OPZ. Methods: In rats treated with intraperitoneal RPZ (20mg/kg or 100mg/kg) or intraperitoneal OPZ (20mg/kg or 100mg/kg) or vehicle for 7 days, intragastric pH, serum gastrin concentration, immunohistochemical positive areas of chromogranin A, fundic histamine content, and mRNA level of HKA were measured. The ultrastructure of parietal cells were also examined by electron microscopy. Results: Both RPZ and OPZ significantly elevated intragastric pH over 6.5 and serum gastrin concentrations, and induced morphological transformation of parietal cells to an active form. Conclusion: These data indicate the effects of RPZ are less than those of OPZ in serum gastrin concentration, positive area of chromogranin A of ECL cells, fundic histamine content, and fundic HKA mRNA level, and in the morphological activation of parietal cells. RPZ does not drive ECL cells and parietal cells so strongly as OPZ in spite of its potent acid suppressive effect and RPZ is the second generation of proton pump inhibitor.

Effects of RPZ and OPZ on ECL cells and parietal cell

		serum G (pg/ml)	Ch A (μm^2)	histamine ($\mu g/g$)	HKA mRNA (%)
vehicle		175±14	998±135	17.6±2.7	100±7.9
OPZ	20mg/kg	1218±108#	270±79#	36.2±5.7*	175±7.8#
	100mg/kg	1495±146#	130±21#	40.2±7.6*	273±6.7#
RPZ	20mg/kg	804±95#	273±89#	20.2±2.7	125±1.9*
	100mg/kg	1102±140#	262±112#	27.3±2.3*	201±18.9#

Data represent mean+-SEM. #p<0.001, *p<0.05 vs vehicle. Serum G: serum gastrin concentration. Ch A represents immunohistochemical positive area of chromogranin A.

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SPECIFIC ACTIVATION OF TE671 CELLS FROM HUMAN CEREBELLUM BY MOTILIN AND MOTILIDES.

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Background. Our laboratory has described the presence of motilin receptors in the central nervous system, in particular in the cerebellum. Aim. To study the response to motilin in a human cerebellar cell line by measuring changes in $[Ca^{2+}]_i$. Methods. The human epithelial TE671 cell line (ATCC CRL-1692), which originates from a cerebellar medulloblastoma, was cultured in coverglass chambers until confluency was reached. Cells were loaded with 10 μM Indo-1-AM and pluronic gel (1/50) dissolved in Krebs (11.6 mM HEPES, 135 mM NaCl, 5.9 mM KCl, 1.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 11.5 mM glucose) for 30 min. at 22°C. Cells were washed and incubated for another 60 min in the absence of Indo-1-AM. For some experiments, 2 μM thapsigargin or 20 μM ryanodine was added during the second incubation. $[Ca^{2+}]_i$ measurements were performed with a confocal laser microscope using an Argon laser operating at 335 nm. Emission was measured at 405 and 470 nm. The proportion of responding cells was calculated after stimulation of the cells with motilin (10^{-5}, 10^{-8} M), the motilin agonist ABT-229 (10^{-5} M), the motilin antagonist OHM-11526 (10^{-5} M), caffein (10 mM) and the calcium channel blocker nifedipine (10^{-6} M). Results are expressed as the mean ± standard deviation of the percentage of the responding cells. Results. Cells responded to motilin after 45 ± 20 seconds with a rapid rise of the fluorescence ratio that slowly declined (90-150 sec). At different concentrations of motilin (10^{-8}, 10^{-7}, $10^{-6.5}$, 10^{-6} and 10^{-5} M) the percentage of cells that responded was 0 ± 0, 0.6 ± 1.5, 4.9 ± 4.7, 21.7 ± 15 and 35.7 ± 12 respectively. The response to motilin 10^{-6} M was blocked by 10^{-5} M of the motilin antagonist OHM-11526 (0.8 ± 1.8%) and mimicked by 10^{-5} M ABT-229 (23.6 ± 15%). After stimulation with motilin, ABT-229 or OHM-11526, cells were desensitised and did not respond when stimulated again. The response to motilin 10^{-6} M (21.7 ± 15%) persisted in Ca^{2+}-free Krebs solution (22.8 ± 14.7%), and was not affected by the calcium channel blocker nifedipine (44 ± 11%). However, incubation with thapsigargin abolished the response (0 ± 0%). Nor ryanodine, nor a previous stimulation with caffein (0 ± 0%) in Ca^{2+}-free Krebs, nor both together could block the response to motilin 10^{-6} M (28%, 32.0 ± 5.7%, 41.3 ± 6.1% respectively). Conclusion. Functional motilin receptors are present in a human cell line of cerebellar origin. In contrast to the situation in smooth muscle cells of the gut, the